Attorney Docket No. 5405-304 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Salvatore V. Pizzo
Serial No.: 10/817,023
Filed: April 2, 2004
Group Art Unit: 1648
Examiner: Emily M. Le
Confirmation No. 2746
For: NOVEL ADJUVANT CAPABLE OF SPECIFICALLY ACTIVATING THE ADAPTIVE IMMUNE RESPONSE

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

DECLARATION OF DR. HERMAN FORD STAATS UNDER 37 CFR §1.132

I, Dr. Herman F. Staats, do hereby declare and say as follows:

1. I am a named inventor on the above-referenced patent application and am familiar with the contents thereof.

2. I have a Ph.D. in Basic Medical Sciences (Microbiology and Immunology) from the University of South Alabama in Mobile, Alabama. I am an Associate Professor in the Departments of Pathology, Immunology, and Medicine at Duke University Medical Center in Durham, North Carolina. I have been conducting research in the area of immunology and vaccines for 20 years and have authored or co-authored more than 119 publications related to this area. A copy of my curriculum vitae is attached as Exhibit A.

3. I have read and understood the Official Action mailed November 28, 2007 (the "Action"), including the rejection of claim 17 for lack of enablement. Claim 17 is directed to a method of inducing an immune response comprising concurrently administering an immunogen and compound 48/80 to a subject in an amount effective to produce an immune response therein, wherein the immunogen and the compound 48/80 are administered simultaneously in a common pharmaceutical carrier, and wherein the immune response is a prophylactic immune response.

4. The studies described below were carried out by me and personnel under my control at Duke University Medical Center according to the disclosure set forth in the '023 application. These studies confirm that administration of an immunogen and Compound 48/80 to a subject induces a prophylactic immune response.

5. The following experimental studies were performed to evaluate the ability of nasal immunization with the poxvirus B5R protein formulated with the mast cell activator Compound 48/80 to induce a protective immunity against a vaccinia virus challenge. Female BALB/c mice (date of birth 7/11/05) were divided into groups of 10 mice each and immunized as indicated in Table 1. Immunizations were initiated on 9/28/05 (mice 11 weeks old). Vaccinia B5R protein was used as the immunogen at 5 μg/dose in phosphate buffered saline for a total of four immunizations per mouse for nasal immunization (20 μg total B5R vaccine per mouse). Serum samples were collected on day 48 and 82 and tested by ELISA for the presence of anti-B5R IgG (Table 2). Vaginal lavage samples were also collected on day 82 and tested by ELISA for the presence of anti-B5R IgG and IgA (Table 2). Only mice immunized with immunogen plus adjuvant had detectable anti-B5R antibody responses. This observation demonstrates that nasal immunization with B5R alone (*i.e.*, in the absence of adjuvant) does not induce the production of B5R-specific antibodies and that the addition of Compound 48/80 to B5R for nasal immunization significantly increases B5R-specific IgG antibodies in the serum of immunized mice.

Table 1. Immunization Groups to Evaluate the Ability of Compound 48/80 to Induce Protective Immunity Against a Nasal Vaccinia Virus, Strain WR Challenge

Group	Adjuvant	Antigen	Route	Volume	Immunization Schedule
1	None	B5R (5 μg)	Nasal	15 μl	Days 0, 7, 14, 49
2	Compound 48/80 (30 μg)	B5R (5 μg)	Nasal	15 μl	Days 0, 7, 14, 49

Table 2. Compound 48/80 is an Effective Adjuvant for Nasally-Administered Poxvirus B5R Protein

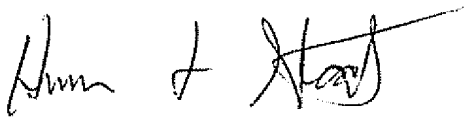
				Serum IgG		Vaginal IgA	Vaginal IgG
Group	**Adjuvant**	**Antigen**	**Route**	**Day 48**	**Day 82**	**Day 82**	**Day 82**
1	None	B5R (5 μg)	Nasal	<1:32	<1:32	<1:4	<1:4
2	Compound 48/80 (30 μg)	B5R (5 μg)	Nasal	1:20,171	1:1,589,344	1:4.3	1:203

6. On day 98, mice were challenged by the nasal route with 1 x 10^5 PFU vaccinia virus WR (Advanced Biotechnologies). Death was not used as an endpoint; mice were considered moribund when they had lost 15% of their bodyweight (based on bodyweight measured at the time of virus challenge) and were humanely euthanized. Mice immunized with B5R immunogen alone were not protected against the nasal vaccinia virus infection and all animals were euthanized by day 6 due to signs of morbidity (Figure 1, Exhibit B). In contrast,

the use of Compound 48/80 as a vaccine adjuvant induced protective immunity since 50% of mice nasally immunized with B5R plus Compound 48/80 were protected against virus-induced morbidity ($p = 0.0325$, Fisher's Exact Test of survival data at day 6).

7. In view of the above, a prophylactic immune response can be induced when Compound 48/80 is administered as an adjuvant with the B5R poxvirus immunogen. I expect, and know of no reason to doubt, that similar prophylactic immune responses will be induced when Compound 48/80 is used as an adjuvant with immunogens from other microorganisms.

16. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



2/16/08

Herman Ford Staats

Date

Figure 1: Compound 48/80 Provides Adjuvant Activity for Nasally-Administered Poxvirus B5R Protein and Provides Protection Against Nasal Infection with 1 x 10^5 PFU Vaccinia Virus WR